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Efficacy of amoxycillin–clavulanate in an experimental model of murine pneumonia caused by AmpC-non-hyperproducing clinical isolates of *Escherichia coli* resistant to cefoxitin

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ABSTRACT

The algorithms included in most automated systems used for antimicrobial susceptibility testing (e.g., Vitek 2) consider that *Escherichia coli* isolates resistant to cefoxitin are AmpC-hyperproducers and, consequently, resistant also to amoxycillin–clavulanate. However, a recent study revealed that 30% of *E. coli* clinical isolates resistant to cefoxitin remained susceptible *in vitro* to amoxycillin–clavulanate. The aim of the present study was to evaluate the in-vivo efficacy of amoxycillin–clavulanate in the treatment of an experimental model of pneumonia, using two clonally related isolates (with identical repetitive extragenic palindromic sequence (REP)-PCR patterns) of AmpC-non-hyperproducing and OmpF-lacking *E. coli* (Ec985 and Ec571) that were resistant to cefoxitin and susceptible to cefotaxime and amoxycillin–clavulanate. MICs were determined using a microdilution technique, and in-vitro bactericidal activity was tested using time-kill assays. The in-vivo efficacy of amoxycillin, amoxycillin–clavulanate and cefotaxime against both isolates was tested in a murine pneumonia model using immunocompetent C57BL/6 mice. Ec571 (a TEM-1/2 producer) was resistant to amoxycillin, whereas Ec985 (a TEM-1/2 non-producer) was susceptible. Amoxycillin, amoxycillin–clavulanate and cefotaxime were bactericidal for Ec985, and amoxycillin–clavulanate and cefotaxime were bactericidal for Ec571 at different concentrations and time-points, as determined using time-kill assays. Treatment with amoxycillin, amoxycillin–clavulanate and cefotaxime reduced the bacterial lung concentration of Ec985 compared with non-treated controls ($p < 0.05$), whereas amoxycillin–clavulanate and cefotaxime showed efficacy against Ec571 when compared with the control and amoxycillin groups ($p < 0.05$). Regardless of the exact underlying mechanism(s) of resistance, amoxycillin–clavulanate was effective in the experimental murine model in the treatment of pneumonia caused by AmpC-non-hyperproducing strains of *E. coli* resistant to cefoxitin.

Keywords Amoxycillin–clavulanate, AmpC-non-hyperproducing, bactericidal activity, *Escherichia coli*, murine model, pneumonia

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INTRODUCTION

Infections caused by *Escherichia coli* are frequently treated with β-lactams and fluoroquinolones. Susceptibility of *E. coli* to β-lactams is variable and may be compromised by β-lactamase production and/or decreased outer-membrane permeability mediated by porin loss [1]. Clinical isolates of *E. coli* that hyperproduce plasmid- or chromosome-encoded AmpC-type β-lactamases are usually resistant to cefoxitin, oxyimino-cephalosporins (i.e., cefotaxime, ceftriaxone and ceftazidime) and amoxycillin–clavulanate, but remain susceptible to zwitterionic cephalosporins (i.e., cefepime and cefpirome) and carbapenems. In contrast, isolates of *E. coli* producing extended-spectrum β-lactamases and porins usually show susceptibility *in vitro* to cefoxitin, amoxycillin–clavulanate and carbapenems. These two

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phenotypes of resistance to β-lactams (AmpC and extended-spectrum β-lactamase production) may be more complex if an additional mechanism of resistance, e.g., porin loss, is present in the same isolate of *E. coli*.

Most automated systems used for antimicrobial susceptibility testing include an expert system or algorithm that is used to interpret phenotypes of resistance/susceptibility to antimicrobial agents. Some of these algorithms (e.g., that used by the Vitek 2 system) consider that isolates of *E. coli* resistant to cefoxitin and susceptible to amoxycillin–clavulanate are AmpC hyperproducers, and consequently, these isolates are assumed to be resistant to amoxycillin–clavulanate. However, a recent study observed that *c.* 30% of clinical isolates of *E. coli* resistant to cefoxitin remained susceptible *in vitro* to amoxycillin–clavulanate [2].

The aims of the present study were to investigate the mechanisms of resistance to β-lactams in clinical isolates of *E. coli* resistant to cefoxitin and susceptible to amoxycillin–clavulanate, and to evaluate the in-vivo efficacy of amoxycillin–clavulanate in the treatment of pneumonia caused by *E. coli* with this phenotype of resistance in an experimental murine model.

MATERIALS AND METHODS

Bacteria

Two isolates of *E. coli* (Ec985 and Ec571), obtained originally from urinary tract infections at the Department of Microbiology, University Hospital Virgen Macarena, Seville, Spain, were used for the study. *E. coli* ATCC 25922 and *E. coli* ATCC 35218 (for antimicrobial susceptibility testing), and *Micrococcus luteus* ATCC 9341 (for the bioassay method) were used as standard reference strains.

Ec985 and Ec571 were identified using the Vitek 2 system (bioMérieux, Marcy l'Etoile, France) and were shown to be clonally related by repetitive extragenic palindromic sequence PCR (REP-PCR) analysis using primers REP-1 (5′-IIIGCGCC GICATCAGGC) and REP-2 (5′-ACGTCTTATCAGGCCTAC) as described previously [3]. The criteria used to select these two isolates were their identical REP-PCR and antimicrobial susceptibility patterns (determined using the Vitek 2 system), except for their susceptibility to amoxycillin (see below).

Antimicrobial susceptibilities

MICs of amoxycillin, cefoxitin, cefotaxime (Sigma-Aldrich, St Louis, MO, USA), cefepime (Bristol-Myers Squibb, Madrid, Spain), imipenem (Merck Sharp & Dohme, Madrid, Spain), meropenem (Zeneca Farma, Madrid, Spain) and amoxycillin–clavulanate (SmithKline Beecham, Madrid, Spain,) were determined by a microdilution assay according to CLSI guidelines [4]. MICs of cefoxitin were also determined in combination with a fixed concentration (4 mg/L) of clavulanate or BRL 42715 (SmithKline Beecham).

Time-kill assays

The bactericidal activities of amoxycillin, amoxycillin–clavulanate and cefotaxime against Ec985 and Ec571 were determined in Mueller–Hinton broth (Becton Dickinson, Cockeysville, MD, USA) using a time-kill assay [5] with an inoculum of 5×10^5 CFU/mL and concentrations of amoxycillin, amoxycillin–clavulanate and cefotaxime that were equal to 1×, 2× and 4× the respective MICs and the C_{max} (maximal serum concentration reached in mice). Tubes with no antimicrobial agent were inoculated with each isolate and used as growth controls. Bacterial counts were determined at 0, 2, 4, 8 and 24 h after incubation (37°C without shaking) by plating 100 μL at the indicated times on sheep blood 5% v/v agar plates in duplicate. An antibiotic was considered to be bactericidal if a ≥ 3 $\log_{10}$ decrease in CFU/mL was observed in comparison with the initial inoculum.

β-Lactamase characterisation

Isoelectric points (pIs) of β-lactamases obtained from crude supernatants were determined by isoelectric focusing using the Phast-System (ampholyte pI range, 3.5–9; Pharmacia, Sant Cugat del Vallés, Spain).

Hydrolysis of cephaloridine (β-lactamase activity) was determined spectrophotometrically using crude extracts. Hydrolysis of cefoxitin was also determined by the Hodge test, modified by replacing the imipenem disk with a cefoxitin (30-μg) disk (Becton Dickinson) [6]. Agar plates were inoculated with a suspension (adjusted to a 0.5× McFarland standard) of *E. coli* ATCC 25922 (indicator strain of cefoxitin hydrolysis).

Mutations in the promoter/attenuator region of the *ampC* gene were identified as described previously [7]. PCR products were sequenced in both directions using a Big Dye Terminator v.3.0 sequencing kit (Applied Biosystems, Foster City, CA, USA). Sequence analysis was performed using a 3700 DNA Analyzer (Applied Biosystems).

Analysis of outer-membrane protein (OMP) profiles

OMPs were analysed by SDS-PAGE (acrylamide 12% w/v and 6 M urea in the running gel), using cell envelopes of sonicated cells as described previously [8].

Mice

Immunocompetent specific pathogen-free C57BL/6 young female mice, weighing 16–20 g, were supplied by the Universidad de Sevilla's animal facility. Animals were housed in regulation cages with free access to food and water. The study was approved by the local Ethical and Clinical Research Committee (approval no. 10/02).

Drug pharmacokinetics

The plasma levels of each drug were determined after the administration of single doses to non-infected mice. Amoxycillin (GlaxoSmithKline, Madrid, Spain), amoxycillin–

clavulanate (GlaxoSmithKline) and cefotaxime (Aventis Pharma, Madrid, Spain) were administered intramuscularly. The dosages used were: 50/5 and 80/8 mg/kg for amoxycillin–clavulanate, 50 and 80 mg/kg for cefotaxime, and 50 mg/kg for amoxycillin. Blood was obtained after 10, 15, 30, 60, 90, 120 and 150 min from an incision in the periorbital plexus of anaesthetised mice, using groups of three mice for each time-point. The drug concentrations in serum were measured by a bioassay method, using *M. luteus* ATCC 9341 as the indicator strain. The maximum plasma concentration (C_{max}; mg/L) and the elimination half-life ($t_{1/2}$; h) were calculated using the PKCALC program [9]. The time during which the plasma concentration remained above the MIC ($T_{>MIC}$, h) was estimated by extrapolation from the regression line of the plasma elimination phase [10], and the percentage of the $T_{>MIC}$ between doses was calculated.

Mouse pneumonia model

The model described by Esposito and Pennington [11], modified by Rodríguez-Hernández *et al.* [12], was used to produce pneumonia in mice. This model was evaluated independently for both Ec985 and Ec571. Mice were infected by intra-tracheal instillation, using 50 μL of a suspension of 5×10^8 CFU/mL in the exponential growth phase, diluted to 50% v/v with porcine mucin (M-2378; Sigma-Aldrich). The experiments were designed to evaluate the decrease in the lung bacterial concentrations after treatments with amoxycillin, amoxycillin–clavulanate and cefotaxime. Taking into account the $T_{>MIC}$ results, the doses and intervals of intramuscular administration were: amoxycillin, 50 mg/kg every 2 h, with five doses; amoxycillin–clavulanate, 50/5 mg/kg every 2 h, with five doses; and cefotaxime, 50 mg/kg every 3 h, with four doses. Control groups without treatment were used for comparisons.

Antimicrobial agents were dissolved in sterile saline (NaCl 0.9% w/v) immediately before administration. The first dose of antimicrobial agent was administered 4 h after the bacterial inoculation (0 h). Beginning at 0 h, immediately before each treatment time-point, three treated and three control mice were killed using an intraperitoneal dose of sodium thiopental 5% w/v (Braun Medical, Barcelona, Spain). The lungs were immediately removed aseptically, weighed, and finally processed for quantitative cultures after homogenisation (Stomacher 8; Tekmar Co., Cincinnati, OH, USA) in 2 mL of sterile saline. After ten-fold dilution, aliquots of 100 μL were plated on sheep blood 5% v/v agar and incubated for 24 h at 37°C. Sterile cultures were considered to indicate ≤1 $\log_{10}$ CFU/g of lung (i.e., the sensitivity limit of the method is equal to 1 CFU). The results were expressed as means ± SDs of the $\log_{10}$ CFU/g of lung values.

Histopathological studies

Lung samples were processed for pathological studies from five mice of each group. The lungs were fixed with formaldehyde 10% v/v, embedded in paraffin, and cut into 4-μm-thick sections. The slices included all the pulmonary lobes, for subsequent study by optical microscopy, and were processed using standard methods for haematoxylin–eosin, periodic acid Schiff's, Gram's, Masson's Trichromic and silver reticulin stains.

Statistical analysis

The CFU/g values for lung tissue among the control, amoxycillin and amoxycillin–clavulanate groups were evaluated by analysis of variance (ANOVA) and post-hoc tests (Dunnett and Tukey tests) for multiple comparisons. The CFU/g values of lung tissue for the control and cefotaxime groups were analysed using the paired Student's *t*-test. Differences were considered to be significant when p values were <0.05. SPSS v.12.0 (SPSS Inc., Chicago, IL, USA) was used for all statistical tests.

RESULTS

In-vitro studies

The two *E. coli* isolates studied were resistant to cefoxitin (MIC 128 mg/L) and susceptible to amoxycillin–clavulanate (MIC 8 mg/L), the broad-spectrum cephalosporins cefotaxime, ceftazidime and cefepime (MICs ≤0.5 mg/L), and the carbapenems imipenem and meropenem (MICs ≤0.06 mg/L). Strain Ec571 was resistant to amoxycillin (MIC 512 mg/L) whereas Ec985 was susceptible (MIC 8 mg/L). The MICs of cefoxitin for both Ec571 and Ec985 decreased from 128 to 64 mg/L in the presence of clavulanic acid or BRL 42715.

Two β-lactamase bands (pIs ≥9 and 5.4, respectively), were observed for Ec571, while Ec985 only had the pI ≥9 band. The β-lactamase activity of the pI ≥9 band was inhibited by cloxacillin, but not by clavulanate. In contrast, the pI 5.4 band was inhibited by clavulanate, but not by cloxacillin. These bands were consistent with the presence of AmpC and TEM-1/2, respectively. Specific β-lactamase activity (mU/mg) was 56.7 for Ec571 and 6.9 for Ec985. Hydrolysis of cefoxitin was not detected spectrophotometrically or with the microbiological method used.

The *ampC* regulatory region of both isolates contained a series of point mutations that were unrelated to AmpC hyperproduction: −88 (C → T), −82 (A → G), −56 (G → C), −18 (G → A), −1 (C → T) and +58 (C → T).

The OMP profiles of the two strains were characterised by the presence of OmpC and OmpA porins, and the absence of OmpF porin.

As summarised in Table 1, the three antimicrobial agents were bactericidal for Ec985 at all concentrations at different time-points, and there was no regrowth. For Ec571 (Table 2) the three antimicrobial agents were bactericidal at all concentrations at different time-points, with the

Table 1. Time-kill assays with amoxycillin (AMX), amoxycillin–clavulanate (AMC) and cefotaxime (CTX) against *Escherichia coli* strain Ec985

		Log_{10} CFU/mL at time (h)				
Antimicrobial agent		**0**	**2**	**4**	**8**	**24**
None (growth control)		6.1	6.7	8.3	9.3	10.3
AMX ($n \times$ MIC)	1×	6.1	5.7	3.7	2.9	1.3
	2×	6.1	5.3	3.7	2.9	1.0
	4×	6.1	4.2	3.7	2.4	1.0
	C_{max}	6.1	2.9	3.7	2.9	1.0
AMC ($n \times$ MIC)	1×	6.1	5.7	5.0	3.2	<1
	2×	6.1	5.7	3.6	2.8	<1
	4×	6.1	4.8	3.0	2.5	<1
	C_{max}	6.1	4.4	2.5	2.5	<1
CTX ($n \times$ MIC)	1×	6.1	5.7	4.8	3.5	2.7
	2×	6.1	5.3	4.4	5.0	2.4
	4×	6.1	5.1	4.1	4.4	2.0
	C_{max}	6.1	4.4	3.7	<1	<1

Table 2. Time-kill assays with amoxycillin (AMX), amoxycillin–clavulanate (AMC) and cefotaxime (CTX) against *Escherichia coli* strain Ec571

		Log_{10} CFU/mL at time (h)				
Antimicrobial agent		**0**	**2**	**4**	**8**	**24**
None (growth control)		6.2	6.8	8.1	9.6	11.3
AMX ($n \times$ MIC)	1×	6.2	3.0	3.0	6.1	11.2
	2×	6.2	<1	<1	3.6	11.0
	4×	6.2	<1	<1	2.7	10.6
	C_{max}	6.2	6.1	7.9	9.4	11.3
AMC ($n \times$ MIC)	1×	6.2	3.7	2.0	2.1	5.6
	2×	6.2	<1	1.8	<1	<1
	4×	6.2	<1	1.6	<1	<1
	C_{max}	6.2	<1	1.3	<1	<1
CTX ($n \times$ MIC)	1×	6.2	3.0	3.5	3.2	5.8
	2×	6.2	<1	3.0	2.0	<1
	4×	6.2	<1	2.9	1.6	<1
	C_{max}	6.2	<1	1.6	<1	<1

exception of the C_{max} of amoxycillin, which was lower than the MIC; moreover, with amoxycillin there was regrowth, beginning at 8 h, at all concentrations tested, and with amoxycillin–clavulanate and cefotaxime at the concentration equivalent to the MIC.

Pharmacokinetics and pharmacodynamics

The serum pharmacokinetic and pharmacodynamic parameters for each antimicrobial agent are summarised in Table 3. For each antimicrobial agent, a dose of 50 mg/kg was chosen for the treatment of the pneumonia because of the similarity of the $T_{>MIC}$ with both 50 and 80 mg/kg.

Histopathological analysis

Mice inoculated with either *E. coli* isolate showed alterations compatible with acute pneumonia, with acute diffuse and/or focal inflammation in all lobes, with mild-to-severe inflammatory infiltration of polymorphonuclear cells, sometimes forming segmentary abscesses, and mild-to-moderate infiltration of alveolar macrophages. Gram-negative bacterial colonies, necrosis and alveolar haemorrhagic areas were also observed.

Table 3. Pharmacokinetic and pharmacodynamic parameters of amoxycillin (AMX), amoxycillin–clavulanate (AMC) and cefotaxime (CTX) in non-infected mice

Agent	**Dosage (mg/kg)**	**MIC (mg/L)**	C_{max} **(mg/L)**	$t_{1/2}$ **(h)**	$T_{>MIC}$ **(h)**
AMX	50	8[a]	15.1	0.28	0.48
AMC	50/5[b]	8[c]	17.9	0.40	0.55
AMC	80/8[b]	8[c]	16.7	0.38	0.49
CTX	50	≤0.5[c]	57.6	0.27	1.89
CTX	80	≤0.5[c]	76.7	0.20	1.57

[a]Isolate Ec985.
[b]AMC doses.
[c]Isolates Ec985 and Ec571.
$T_{>MIC}$: time with serum concentrations above the MIC.

Bacterial clearance from lungs

The bacterial concentrations in the lungs of the different treatment groups are summarised in Tables 4 and 5. For Ec985, treatment with amoxycillin, amoxycillin–clavulanate and cefotaxime significantly reduced the bacterial concentration in the lungs ($p < 0.05$). The highest rate of bacterial clearance for Ec985 was observed with amoxycillin–clavulanate (−3.81 log_{10} CFU/g at 8 h). In comparison with the control groups, the differences in bacterial counts with the three treatments were also significant ($p < 0.05$). No differences among the treatment groups were revealed.

For Ec571, treatment with amoxycillin–clavulanate and cefotaxime reduced the bacterial concentration in the lungs ($p < 0.05$), with no significant difference between the two agents. However, amoxycillin was ineffective in reducing the number of bacteria. Again, the highest rate of bacterial clearance was observed with amoxycillin–clavulanate (−3.40 log_{10} CFU/g at 8 h).

DISCUSSION

The two clonally related isolates of *E. coli* included in the present study produced an AmpC β-lactamase (pI ≥9), but did not hyperproduce it, based on the high level of susceptibility of both strains to cefotaxime and ceftazidime, the low level of β-lactamase activity detected, and the absence of

Table 4. Bacterial concentrations (mean $\log_{10}$ CFU/g of tissue ± SD) in the lungs of mice infected with *Escherichia coli* isolates Ec985 and Ec571, either without treatment (Control) or treated with amoxycillin (AMX) or amoxycillin–clavulanate (AMC)

	Time (h)					
	0	2	4	6	8	10
Ec985						
Control	8.94 ± 0.39	9.91 ± 0.97	8.40 ± 1.51	8.26 ± 1.33	8.47 ± 0.53	8.99 ± 0.57
AMX	8.94 ± 0.39	8.33 ± 0.25	6.91 ± 0.46[a]	7.12 ± 0.05[a]	7.01 ± 0.19[a]	6.82 ± 0.41[a]
AMC	8.94 ± 0.39	7.84 ± 0.34[a]	7.13 ± 0.34[a]	5.40 ± 0.18[a,c]	5.13 ± 0.57[a,b,c]	6.34 ± 0.13[a,b]
Ec571						
Control	8.43 ± 0.83	9.10 ± 0.07	8.46 ± 0.69	8.57 ± 0.65	9.30 ± 0.38	9.72 ± 0.09
AMX	8.43 ± 0.83	9.29 ± 0.19	8.20 ± 0.09	8.83 ± 0.30	9.43 ± 0.12	8.81 ± 1.57
AMC	8.43 ± 0.83	6.99 ± 0.52[b,c]	6.30 ± 0.06[a,c]	5.83 ± 0.04[a,b,c]	5.03 ± 0.03[a,b,c]	5.54 ± 0.40[a,b]

[a]$p < 0.05$ in intra-group comparisons with respect to bacterial concentrations at time 0 h.
[b]$p < 0.05$ compared with control group.
[c]$p < 0.05$ compared with AMX group.

Table 5. Bacterial concentrations (mean $\log_{10}$ CFU/g of tissue ± SD) in the lungs of mice infected with *Escherichia coli* isolates Ec985 and Ec571, either without treatment (Control) or treated with cefotaxime (CTX)

	Time (h)				
	0	3	6	9	12
Ec985					
Control	8.79 ± 0.48	9.30 ± 0.06	8.26 ± 1.33	8.94 ± 0.17	9.16 ± 1.44
CTX	8.79 ± 0.48	7.67 ± 0.23[a,b]	6.62 ± 0.38[a]	5.88 ± 0.37[a,b]	6.12 ± 0.28[a,b]
Ec571					
Control	8.43 ± 0.91	9.07 ± 0.22	8.57 ± 0.65	9.53 ± 0.13	7.83 ± 1.79
CTX	8.43 ± 0.91	6.63 ± 0.03[a,b]	5.81 ± 0.13[a,b]	5.58 ± 0.53[a,b]	5.64 ± 0.17[a]

[a]$p < 0.05$ in intra-group comparisons with respect to bacterial concentration at time 0 h.
[b]$p < 0.05$ compared with control group.

hydrolysis of cefoxitin. Additionally, most of the point mutations detected in the regulatory region of *ampC* have been reported previously to be unassociated with hyperproduction of AmpC [7]. Although the effect of mutation at position –56 has not been studied previously, the low level of β-lactamase activity in both isolates suggests that it may not have a significant role in the hyperproduction of AmpC. Previous studies have suggested that resistance of AmpC-hyperproducing isolates of *E. coli* to cefoxitin may be achieved by the loss of the OmpF porin [8]. Since the two isolates of *E. coli* in the present study lacked OmpF, but were not hyperproducers of AmpC, it is possible that additional mechanisms (e.g., overproduction of efflux pumps or alterations in penicillin-binding proteins) might combine with the lack of OmpF to produce resistance.

Whatever the actual underlying mechanism of resistance, amoxycillin, amoxycillin–clavulanate and cefotaxime had a significant bactericidal effect *in vitro* against the isolate (Ec985) that did not produce TEM-1/2 β-lactamase, suggesting that these antimicrobial agents could also be active *in vivo*. For the isolate producing TEM-1/2 (Ec571), amoxycillin–clavulanate and cefotaxime were also bactericidal *in vitro*, indicating that production of TEM-1/2 has no effect *in vitro* on the activity of these antimicrobial agents.

The study also investigated whether amoxycillin–clavulanate could be used to treat infections caused by *E. coli* with the novel phenotype of resistance to cefoxitin in the absence of AmpC overproduction. The experimental model of pneumonia in immunocompetent mice used in this study is simple to develop and yields highly reproducible results [12]. The main limitation of this model is that *E. coli* is not among the most common causes of pneumonia. However, *E. coli* causes 4.3% of cases of severe community-acquired pneumonia, with a mortality rate of 35% [13], and also causes late-onset nosocomial pneumonia [14].

The in-vivo experiments revealed that amoxycillin–clavulanate is as effective as cefotaxime in the treatment of pneumonia caused by *E. coli* with the novel resistance phenotype. The same degree of efficacy was observed despite the higher $T_{>MIC}$ between doses of cefotaxime (63%) and amoxycillin–clavulanate (27%). Amoxycillin, with a $T_{>MIC}$ (24%) similar to that of amoxycillin–clavulanate, was effective against pneumonia caused by Ec985 (susceptible to amoxycillin), but not against that caused by Ec571 (resistant to amoxycillin).

β-Lactams have a time-dependent bactericidal effect and, in order to maximise their activity, the $T_{>MIC}$ should remain close to 40% [15]. The $T_{>MIC}$ for amoxycillin and amoxycillin–clavulanate in the present study was lower (*c.* 25% between doses) because of the choice, for ethical reasons, of a minimum interval between doses of 2 h. However, no reduction in efficacy was observed for either antimicrobial agent in the treatment of experimental pneumonia caused by isolates showing in-vitro susceptibility.

In conclusion, these results indicate that isolates of *E. coli* resistant to cefoxitin and susceptible to amoxycillin–clavulanate should be checked for AmpC hyperproduction to avoid erroneous reporting of resistance to amoxycillin–clavulanate. It appears that loss of porin OmpF, but not hyperproduction of AmpC, is partially responsible for the resistance pattern of such isolates. Finally, although the in-vivo experiments suggested that amoxycillin–clavulanate could be used for the treatment of infections caused by *E. coli* with this resistance phenotype, additional in-vivo experiments should be performed before definitive clinical recommendations can be made.

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